

### Abstract

*Vernonia anthelmintica* (L). Willd. (ironweed) seed from Pakistan or India produced 22–28% oil rich in trivernolin, the glyceride of 12,13-epoxyoleic acid, when the highly active lipolytic enzyme was properly controlled. Trivernolin was obtained from the seed as a major end-product without isolation of the oil from the extract in yields of 55–60% of the wt of the oil present. Control of lipolysis was achieved, either by a rapid extraction technique where whole seed was flaked as a slurry made of seed and extracting solvent (petroleum naphtha, bp 35–59C), or by autoclaving the seed prior to flaking or grinding. An improved quality oil was produced by removal of the major portion of the unsaponifiable material; this upgraded the oil by increasing the epoxy lipid content, decreasing the iodine value (I.V.) and reducing the amount of color and odor.

### Introduction

DEVELOPMENTAL RESEARCH ON *V. anthelmintica* at this laboratory is a part of an extensive USDA project which seeks new cash crops especially to relieve or replace those in surplus. The seed of this species of *Vernonia*, native to India and Pakistan, yields 22–28% oil containing about 70–75% vernolic (12,13-epoxyoleic) acid (Fig. 1) combined as glycerides. A previous report (1) described the epoxy fatty components isolated from the seed oil and presented a comprehensive review of the literature on this species of *Vernonia*. *V. anthelmintica* is not now a large commercial crop and considerable agronomic development will be required to make growing on a large scale feasible. However, good quality seed has been grown in this country during the 1963 season at a number of locations. This report deals with the production of trivernolin-rich oil from *V. anthelmintica* seed and with a simple procedure for obtaining pure trivernolin as a major product without isolation of the oil from the extract; the report also presents a method for the removal of the unsaponifiable material which usually amounts to ca. 6–7% (1,2) of the wt of the oil, thereby improving the quality of the oil.

### Experimental Procedures and Results

**Materials and Equipment.** The *Vernonia* seed used in these investigations was from India or Pakistan, supplied in part through the courtesy of Quentin Jones, Crops Res. Div., USDA; a portion was purchased from Herbst Brothers, Seedsmen, Inc., New York, N. Y. Analytical data on seed samples and on the prepared products were obtained as previously described (1).

In the small-scale laboratory experiments, either a Waring Blendor or a "FitzMill" was used for comminuting the *Vernonia* seed. The W. J. Fitz-

patrick Comminuting Machine (Chicago, Ill.) was equipped with a No. 2 screen ( $\frac{1}{16}$  in. diam holes). In the FitzMill, dry seed was comminuted and immediately placed in the solvent (n-hexane). Seed was pulverized in the Waring Blendor in the presence of solvent (petroleum naphtha, p.n., bp 35–59C). In both procedures, extracts were decanted through a suction filter equipped with a No. K-5 (Republic-Seitz Filter Corp., Newark, N. J.) filter pad. Solvent was removed by a rotating evaporator under reduced pressure.

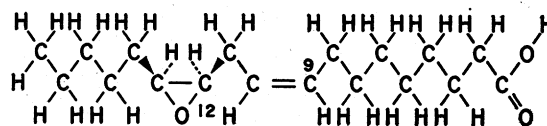
In the larger-scale experiments using from 10–67.6 lb *Vernonia* seed with p.n. as the solvent, the seed was flaked in a Model NSP mill (Lauhoff Rolling Mill Corp., Detroit, Mich.).

When whole dry *Vernonia* seed was flaked it was fed to the rolling mill by a Vibro-Flow Feeder (Type FMO 10) manufactured by the Syntrol Company of Homer City, Pa.; the rate of feed was ca. 1–2 lb/min. When solvent-wetted *Vernonia* seed was flaked the slurry was fed manually to the rolling mill at the rate of ca. 2 lb/min. In either case flaked seed dropped into a 40-gal receiver partially filled with solvent. Cakes of dry-ice were placed over the flaking rollers for the purpose of creating a safe working atmosphere with p.n. After flaking, sufficient solvent was added to bring the ratio to about 2–3 gal/lb seed for a first extract. The mixtures were stirred for ca. 3 min, the mares allowed to settle, and the supernatant extracts pumped off through a Sparkler filter press equipped with No. K-5 filter pads to 20-gal evaporators for removal of solvent under reduced pressure.

To clarify dark colored oils or trivernolin, as much as 1% Darco G-60 and 2% Filtrol No. 4 based on the quantity of seed extracted was added during the stirring process in the extraction of flaked seed. When mares were used for experimental animal feeding the clarification step was added after the initial filtration of extracts and washings and a second filtration performed to remove the adsorbents from the miscella.

Where enzyme activity was controlled by autoclaving, the whole seed was treated at optimum conditions (3) of ca. 15 psi, 120–125C, for 30–45 min.

**A. Small-Scale Laboratory Extractions.** The purpose of these small-scale extractions was to supplement the rapid extraction technique previously described (1) to find out if this procedure for the con-



D-(+)-cis-12,13-epoxy - cis-9-octadecenoic acid  
(12,13-epoxyoleic)  
 $C_{18}H_{32}O_3$

Fig. 1. Vernolic acid formula.

<sup>1</sup> Presented at the AOS meeting, Minneapolis, 1963.

<sup>2</sup> E. Utiliz. Res. and Dev. Div., ARS, USDA.

TABLE I  
Extraction of FitzMill Comminuted *Vernonia* Seed  
(417.3-g samples, mfb, oil 26.4%)  
Experiment 1. For oil using eight 3.6-l portions of n-hexane at 4C

Extract No.	Oil			
	Quantity		Free fatty acids (FFA) calcd as epoxyoleic	Oxirane oxygen
	g	%	%	%
1-3.....	99.6	23.9	2.5	3.78
4-5.....	1.5	0.36	9.3	3.00
6-8.....	0.7	0.16	12.0	2.67
Total.....	101.8	24.4		

Experiment 2. For trivernolin using one 3.6-l; two 0.9-l portions (combined) of n-hexane at 4C

Crop No.	Trivernolin				
	Quantity		Free fatty acids (FFA) calcd as epoxyoleic	Oxirane oxygen	Purity
	g	%	%	%	%
1.....	45.4	10.9	0.6	5.04	97.4
2.....	8.2	2.0	0.7	4.70	90.8
Residue.....	34.0	8.1	5.3	2.03	39.2
Total.....	87.6	21.0			

trol of lipolytic activity in *Vernonia* seed might be feasible for larger-scale operations. In the work previously described, the seed was ground in a Wiley mill to pass a screen with 2-mm diam holes; finer screens could not be used because of the oily character of the seed and the overheating of the mill. The free fatty acid content of the oil was low (1.9%) if the extraction was quickly completed (less than 90 min). However, this procedure failed to extract all of the oil; for example, only 20.7% compared to an analytical figure of 23.9% for one oil. When the marc was dried, reground to pass 0.5-mm diam holes and exhaustively extracted in a Soxhlet apparatus an additional 2.9% of oil was obtained. In replicas of this experiment where the time consumed in grinding and extracting was increased to 3 hr the free fatty acid content of the oils produced was increased from 1.9-3.5%. Two-hr extraction periods in a Soxhlet have produced oils with a free fatty acid content of 8% and higher.

Two experiments (Table I) are representative of optimal trials with the FitzMill preparation of seed for extraction, experiment 1 for oil and experiment 2 for trivernolin. In experiment 1, *V. anthelmintica* seed (417.3 g mfb, oil 26.4%) and n-hexane were cooled to 4C. The seed was comminuted in a pre-cooled (dry-ice) mill and immediately placed in 3.6 liters solvent with stirring. The temp was maintained at 4C with dry-ice. The extract was removed by decantation and filtered by suction through a K-5 pad. The marc was re-extracted 7 times with 3.6-l portions of cold solvent. Extracts 1-3, 4-5 and 6-8 were combined and worked up separately for oil with results as shown in Table I.

In experiment 2 (Table I), the same quantity of seed was prepared and extracted as in experiment 1 except that 9.0 g (ca. 10% of the wt of oil present in the seed) of Filtrol No. 4 and 4.5 g Darco G-60 was stirred with the 3.6-l solvent-seed mixture in prepar-

TABLE II  
Waring Blendor Extraction of *Vernonia* Seed, 27.2% Oil  
(46.0-g, mfb, Samples. Eight 400-ml p.n. extractions)

Experiment No.	Extraction		Oil			
	Time	Temp	Quantity		FFA	Oxirane oxygen
	hr	°C	g	%	%	%
1 <sup>a</sup> .....	1.5	25	12.1	26.3	1.4	3.88
2.....	6.0	25	11.8	25.8	3.6	3.86
3.....	6.0	4-8	12.8	27.9	1.3	3.92

<sup>a</sup> Eight 200-ml p.n. extractions.

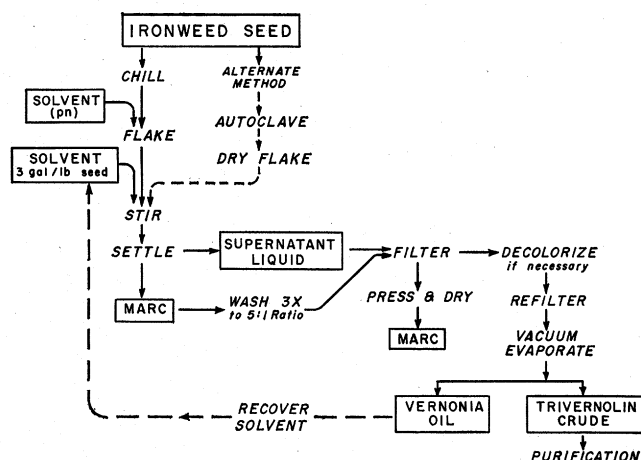


Fig. 2. Production of *Vernonia* seed oil.

ing the initial extract. Also, the marc was extracted only twice with 0.9-l portions of cold n-hexane and these two were added to the initial extract and the combination was then evaporated to 1.8 liters. This concentrate was chilled to -20C with mechanical stirring to obtain trivernolin. In 2 hr the trivernolin crystals were removed at -20C on a filter plate and washed 3 times with minimal quantities of cold solvent. The trivernolin was dried to a constant wt of 45.4 g in a rotating evaporator; the yield was 10.9% (purity 97.4%) based on the dry wt of seed used; the product was an almost water-white liquid, Gardner No. less than one. This yield compares favorably with the 11.7% previously reported (1). A second crop (8.2 g) of trivernolin of lower purity (90.8%) was obtained by concn of the mother-liquor to 0.9 liters and chilling to -20C. Complete removal of solvent produced a residue of 34.0 g; total solids extracted was 87.6 g (21.0%). Only 2.6 g (less than 1%) oil was obtained by 3 additional extractions of the marc at 4C using 0.9-liter portions of solvent.

The three Waring Blendor experiments presented in Table II serve to illustrate: that optimum extraction occurred at the temp range 4-8C with respect to quantity and quality of oil, and that at the lower temp enzyme activity was reduced to a min and extreme haste in extraction procedures was unnecessary when seed was comminuted wetted with the solvent.

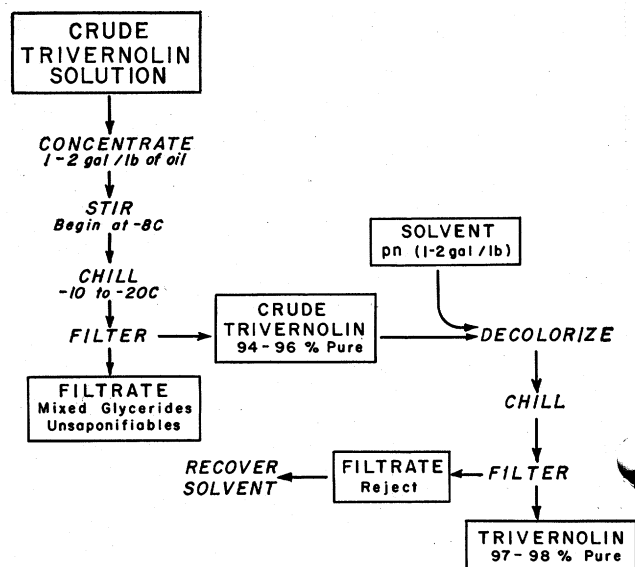


Fig. 3. Isolation and purification of trivernolin.

TABLE III  
V. anthelmintica Seed Extractions. Rapid Extraction for Oil

Experiment No.	Quantity of seed	Moisture in seed	Oil in seed	Chief variable in procedure	Extract No.	Solvent used	Oil			
							Quantity		FFA as epoxy-oleic	Iodine value (Wijs)
	kg	%	%			gal	g	%	%	
1.....	26.6	7.7	22.0	Seed (dry) flaked at 25-30C	1	30	4187	17.3	3.4	104.2
					2	25	942	3.9	10.1	105.1
					3	25	131	0.54	21.4	106.5
					4	25	55	0.23	26.1	109.0
					5	15	20	0.08	31.3	105.3
					Total	120	5335	22.0		
2.....	4.53	8.0	27.2	Seed (wet) flaked at 10-16C	1	30	899.9	21.6	1.0	107.0
					2	5	73.2	1.8	3.9	105.0
					3	3 <sup>a</sup>	22.5	0.54	4.1	84.9
					4	7 <sup>a</sup>	37.3	0.89	5.3	99.5
					5	6 <sup>a</sup>	21.3	0.51	6.8	90.6
					Total	51	1054.2	25.3		
3.....	4.53	8.0	27.2	Repeat Expt 2 using hot extraction	1	30	895.4	21.5	1.1	104.4
					2-4	20 <sup>a</sup>	100.6	2.4	1.92	70.6
					Total	50	996.0	23.9		

<sup>a</sup> New p.n. used; contaminated with kerosene.

In the Waring Blendor experiments 50.0-g (46.0-g mfb) samples of whole seed (oil by exhaustive Soxhlet extraction, 27.2%) were used. Seed and p.n. were ground together at high-speed for 3-min periods; the mixture was allowed to settle, and the supernatant liquid was decanted through a K-5 filter pad. In the first experiment, each of 8 extracts was worked up independently for oil (break-down not shown in Table II) which demonstrated that about seven extracts had to be made to obtain all of the oil.

B. Larger-Scale Extractions. This section is divided into four parts: 1) rapid technique for oil; 2) autoclaved seed for oil; 3) rapid technique for trivernolin; and 4) autoclaved seed for trivernolin. All of the illustrations presented represent selected experiments. *Vernonia* products made in these, and in other extractions not detailed, were used for our own and industrial evaluation studies. In these extractions enzyme activity was controlled either by the use of a rapid extraction technique, where whole seed wetted with sufficient solvent to make a slurry, was flaked in the rolling mill, or by autoclaving whole seed before flaking. Autoclaving was previously reported (3) to be superior to the use of dry heat for inhibiting enzyme activity. Trivernolin-rich oil and trivernolin were produced by both of these procedures (see schematic diagrams, Fig. 2,3).

1) Rapid technique for oil. Three experiments presented in Table III illustrate this procedure. Experiment 1 was included to show that seed flaked dry at room temp could not be handled rapidly enough to prevent objectionable lipolytic action. In this experiment 58.5 lb (26.6 kg with 7.7 % moisture content) *Vernonia* seed (oil by exhaustive Soxhlet technique, 22.0%) was flaked dry at a working temp of 25-30C. The flaked seed coming off the mill rollers dropped immediately into a tank containing the p.n. extracting solvent. The mixture was diluted to 30 gal, stirred for ca. 3 min, allowed to settle for ca. 3 min and the supernatant extract pumped off through the filter press to the evaporators; the solvent was removed at once. A second extract was made immediately, using 25 gal solvent and the same procedure; data on these and additional extracts show in Table III. Most of the oil was extracted in the first three extracts. However, the oil was not of satisfactory quality as indicated by the free fatty acid percentages of the extracts shown in Table III.

In experiment 2, illustrated in Table III, 10 lb (4.53 kg with 8.0% moisture content) *Vernonia* seed (oil 27.2%) was wetted with ca. 2-3 gal (sufficient to make a slurry) of p.n. This wet mixture was

flaked and allowed to drop into the receiving tank containing solvent and the extracts handled as described in experiment 1. Most of the oil was obtained in the first two extracts, 30 and 5 gal, respectively; ca. 3.5 gal of p.n./lb seed. Oil from the first extract was highly satisfactory with respect to quantity, low free fatty acid and high oxirane oxygen content. The lower temp (10-16C), supplied by winter weather in an unheated building, was conducive to retarded enzyme activity in agreement with the small-scale Waring Blendor experiments.

In experiment 3, the second experiment was repeated with the exception that following dilution after flaking, the mixture was transferred to a steam-jacketed tank with a false-bottom and heated during recycling for a period of three min. Extracts 2-4 were made with fresh boiling p.n. in similar fashion, combined and worked up together. No advantages over the cold extraction were gained by the hot extraction procedure.

2) Autoclaved seed for oil. A single experiment (Table IV) has been selected for illustration in which optimum conditions for autoclaving the seed were used. The 22.5 lb (10.2 kg, moisture 8.0%, oil 27.2%) whole autoclaved *Vernonia* seed was flaked (not wetted with solvent) in the rolling mill equipped with automatic feed on the day following the heat treatment. Extracts were made in the manner described for the "rapid technique" using the quantities of solvent shown in Table IV. Data for 8 extracts showed that lipolysis was adequately controlled during the operation. Since exhaustive extraction had not been obtained, two additional extractions of the marc were made one week later. Small quantities of oil were obtained with increased free fatty acid content, indi-

TABLE IV  
V. anthelmintica Seed Extractions. Autoclaved Seed for Oil (10.2 kg seed containing 8.0% moisture, 27.2% oil)

Extraction		Oil <sup>a</sup>			
No.	Solvent	Quantity		FFA as epoxy-oleic	Oxirane oxygen
		gal	%	%	%
1.....	20	1673.4	17.8	0.8	3.85
2.....	5	353.5	3.76	1.0	3.86
3.....	5	121.5	1.29	1.2	3.82
4.....	5	69.0	0.73	1.4	3.72
5.....	5	59.7	0.64	1.4	3.82
6.....	5	52.0	0.55	1.6	3.77
7.....	5	25.4	0.27	1.8	3.73
8.....	7	56.2	0.60	1.4	3.74
9 <sup>b</sup> .....	5	52.5	0.56	3.8	3.27
10 <sup>b</sup> .....	5	47.0	0.50	3.1	3.53
Total.....	67	2510.2	26.7		

<sup>a</sup> Iodine values were obtained but were not recorded since they were between 103 and 105.

<sup>b</sup> Marc re-extracted after 7-day storage.

TABLE V  
*V. anthelmintica* Seed Extractions. Rapid Extraction for Trivernolin<sup>a</sup>  
 (7.32 kg of seed containing 8.0% moisture, 27.2% oil)

Extraction		Fractions obtained by crystallization at -20C						
No.	Solvent	From crop No.	Quantity		Oxirane oxygen	Trivernolin purity	FFA as epoxyoleic	Iodine value (Wijs)
	gal		g	%	%	%	%	
1.....	30+5	1	864.2	12.80	5.00	96.6	0.50	83.9
		2	53.9	0.80	4.79	92.5	0.43	84.6
2.....	30	1	74.1	1.10	4.76	92.0	14.7	86.1
3.....	40	1	46.9	0.69	4.96	95.8	39.0	85.1
		2 <sup>b</sup>	28.8	0.43	4.57	88.9	0.74	83.5
		Residue	698.5	10.37	2.21	42.7	7.93	128.9
Total.....	105		1766.4	26.2				

<sup>a</sup> Yield based on oil, 56.3% (96.0% pure) (15.4% based on the seed) using extracts 1-3 for calculation.

<sup>b</sup> All mother-liquors from extracts concn to ½ gal.

cating either selective extraction or that the hydrolytic enzyme was not completely inactivated by autoclaving under the conditions of the experiment.

3) Rapid technique for trivernolin. One selected example of the preparation of trivernolin using the same procedure as previously described under the heading "1. Rapid technique for oil" shows in Table V. About 15 lb (7.32 kg, moisture 8.0%, oil 27.2%) seed was wetted with solvent before flaking and extracting at 10-16C. A 30-gal extract was made and a quick 5-gal wash of the marc was added to the extract. After filtering no solvent was removed; the entire solution containing the extracted oil was placed in the refrigerator at -20C. During crystallization which started at -8C, the mixture was mechanically stirred. After removal of the crude trivernolin by filtration at -20C, the filter cake was washed three times by removing it from the filter-plate each time, slurried with fresh cold solvent, mechanically stirred, and refiltered. Concn of the mother liquor and washings to 3 gal produced a second crop of trivernolin (53.9 g) in addition to the original 864.2 g. Additional extractions of the marc were made to obtain exhaustive extraction of the trivernolin; the number of extracts made and the quantities of solvent used show in Table V. Extracts 1-3, when combined, gave a trivernolin yield of 56.3% of the wt of oil present in the seed with a purity of 96.0% based on the oxirane oxygen percentage. The extract marked "residue" in Table V was obtained by evaporation of all mother liquors and was composed chiefly of mixed fatty acid glycerides (for fatty acid composition of *V. anthelmintica* seed oil see Reference 1) and unsaponifiable material.

4) Autoclaved seed for trivernolin. A single experiment illustrates the use of autoclaved seed in the production of trivernolin. Information on the seed and the quantities of solvent used show in Table VI. Flaking and extraction procedures were similar to those described under "2. Autoclaved seed for oil." However, only partial crystallization of trivernolin occurred (376.5 g obtained) when the entire first

extract of 35 gal was chilled to -20C. To obtain a second crop (311.9 g) the mother-liquor was evaporated to 1.5 gal and chilled to -20C. Concn of the second crop mother-liquor produced 37.8 g trivernolin of inferior quality. The 20-gal extracts, 2 and 3, of the marc were concn to 3 gal before cooling.

C. Production of Vernonia Oil of Improved Quality. The process used to obtain an enriched oil with respect to epoxy fatty components consisted of the freezing out of the lipid glycerides from a p.n. solution with a ratio of ca. 3 ml solvent/g oil at -60C. The solids were removed by vacuum filtration at -60C and washed several times with minimal amounts of cold solvent; when melted at room temp, traces of solvent were removed in a rotating evaporator under vacuum. Table VII presents data obtained in this operation. Improvement in quality of the oil is indicated by the increase in oxirane oxygen content from 3.85-4.38%, a decrease in the I.V. from 104.1-87.8 and a decrease in the quantity of unsaponifiable material from 6.67-1.20%. Compositional studies of the unsaponifiable material are in progress.

## Discussion

Results obtained in the supplemental small-scale extractions established the feasibility of the rapid extraction procedure for larger-scale operations and indicated the desirability of the use of lower temp to retard lipolytic enzyme activity; also, the superiority of the use of solvent-wetted seed in the crushing operation. Although flaking was the preferred procedure for crushing *Vernonia* seed, preliminary experiments with the FitzMill indicated the possibility of the use of a mill of this type for large-scale production if lower oil yields would be acceptable.

In the larger-scale operations, the flaking technique, using whole seed wetted with solvent prior to rolling, produced the best yields of high quality oil. The lower temp supplied by the winter working conditions were also conducive to the inhibition of enzyme activity. No advantage was gained by the use of hot solvent following the flaking operation. The technique

TABLE VI  
*V. anthelmintica* Seed Extractions. Autoclaved Seed for Trivernolin<sup>a</sup>  
 (6.68 kg of seed containing 8.6% moisture, 22.1% oil)

Extraction		Fractions obtained by crystallization at -20C						
No.	Solvent	From crop No.	Quantity		Oxirane oxygen	Trivernolin purity	FFA as epoxyoleic	Iodine value (Wijs)
	gal		g	%	%	%	%	
1.....	30+5	1	376.5	6.17	4.99	96.4	0.34	80.8
		2	311.9	5.12	4.83	93.3	0.38	80.6
		3	37.8	0.62	4.54	87.7	0.61	82.6
2.....	20	1	24.8	0.41	4.78	92.3	1.9	83.6
3.....	20	1	2.0	0.03	4.49	86.7	2.0	.....
		Residue	588.2	9.63	2.02	39.0	2.3	148.8
Total.....	75		1341.2	22.0				

<sup>a</sup> Yield based on oil, 51.3% (95.0% pure) (11.3% based on the seed) using extracts 1 and 2 for calculation.

TABLE VII  
Crystallization of Lipid Glycerides of *V. anthelmintica* Seed Oil at  
-60C for Removal of Unsaponifiable Material

	Quantity	Oxirane oxygen	FFA as epoxy- oleic	Iodine value (Wijs)	Unsaponi- fiable material
	g	%	%		%
Oil.....	1698	3.85	0.86	104.1	6.67
Precipitate.....	1511	4.39	0.52	87.8	1.20
Filtrate.....	188	0.29	2.4	190.0	53.0

of flaking dry seed at room temp, followed by rapid handling in the extraction steps, was not satisfactory from the standpoint of producing an oil with a low free fatty acid content. Even when the flaked seed was allowed to fall directly from the rollers of the mill into the solvent this was not successful in holding down lipolysis.

The process of autoclaving the seed to inhibit lipolysis prior to flaking also was a successful procedure in oil production as illustrated by the fractional extraction data obtained. The slight variation in the free fatty acid content and oxirane oxygen composition of the 10 extracts may have been due to selective extraction of the epoxy fatty components. When the seed was autoclaved no haste was necessary in carrying out extraction operations.

In situations where trivernolin was the desired end-product (Tables V and VI) the rapid technique offered several advantages over the autoclaved seed treatment: the yield of good-quality trivernolin was

higher; less coloring material was extracted; and it was not necessary to concn the solvent before crystallization in order to obtain the product in good yield. The solubility of trivernolin has previously been reported (4) as ca. 5 g/gal at -20C.

No plastic formulation data are available on the evaluation of *Vernonia* oil where the major portion of the unsaponifiable material has been removed. As this process appreciably elevated the oxirane oxygen percentage, reduced the I.V., and removed color and odor, it may have resulted in an improved product for the purpose stated.

Since the quantity of oil extracted by Waring Blendor technique, 27.9%, is in close agreement with the analytical figure of 27.2% obtained by tedious, exhaustive Soxhlet extraction (1) the former method is now recommended for estimation of the quantity of oil in *Vernonia* seed. Additional experiments have confirmed the utility of this procedure.

#### ACKNOWLEDGMENTS

Consultation with R. W. Riemenschneider; outstanding technical assistance and performance in these studies by Fred J. Oelshlegel, Jr., student trainee.

#### REFERENCES

1. Krewson, C. F., J. S. Ard, and R. W. Riemenschneider, *JAOCS* 39, 334-40 (1962).
2. Gunstone, F. D., *J. Chem. Soc.*, 1611 (1954).
3. Scott, W. E., C. F. Krewson, F. E. Luddy, and R. W. Riemenschneider, *JAOCS* 40, 587-589 (1963).
4. Krewson, C. F., and F. E. Luddy, *JAOCS* 41, 134-136 (1964).

[Received November 18, 1963—Accepted January 23, 1964]